Supplementary information (Nature. #2006-11-12261F)

Methods

ESC Lines and Culture Conditions

mESCs (E14Tg2A) obtained from Bay Genomics, were cultured under self-renewal conditions (in the presence of leukemia inhibitory factor, LIF, cultured on adherent tissue culture dishes) without feeder cells in 1000 units ml^{-1} LIF (ESGRO) on gelatin-coated tissue culture dishes[1]. Embryoid bodies in absence of LIF and b-mercaptoethanol on non-adherent tissue culture dishes were grown for 3 days in bacterial dishes as described[2]. The *REST*$^{+/-}$ cell lines YHC334 (YHC) and RRC160 (RRC) that have gene traps at *REST* exons 1 and 2, respectively were obtained from BayGenomics. Both of these lines were derived from parental E14Tg2A mES cells, the same cells we used for all of our wild-type mES cell experiments.

ChIP Assays

ChIP assays of cultured mESCs and EBs were performed as described [3]. We used antibodies against mouse IgG (2 μl; cat. no. I 5381; Sigma), REST (2μl ; cat. no. 07-579, Upstate) and histone H3 (2 μl; cat. no. ab1791; Abcam). One microgram of each antibody and approximately 15 μg of chromatin were added to each reaction mixture and incubated overnight at 4 °C. The mixtures were then incubated with 33 μl of protein A-agarose beads (Amersham Pharmacia Biotech AB; pre-equilibrated with 1 mg ml^{-1} bovine serum albumin and 0.3 mg ml^{-1} salm on sperm DNA).

The PCR primer sequences used are as follows:

miR-93/miR-106b Forward: cccccaaaaccagtatcctt
miR-93/miR-106b Reverse: tcgtacttcccggatcactc
miR-27a Forward: aaatccccagaagctggagt
miR-27a Reverse: agcacttgaaaggcaaagga
miR-21 Forward: agggcaggaagatgacacac

miR-21 Reverse: gggctgatgagcactaagga
miR-26a Forward: gcctaacccaagaagggaaa
miR-26a Reverse: tccctctcatctggacaacc
miR-152 Forward: tggtctctgtccagcacaac
miR-152 Reverse: caggtcacagctgcactcat
miR-124a Forward: ctctgcgtgttcacagcgg
miR-124a Reverse: ctcttggcattcaccgcgtg

Quantitative ChIP PCR was performed using the same extracts used above. Briefly, QPCR was performed and analyzed using real-time PCR (ABI prism 7500). Primers were designed by Primer Express 2.0 (Applied Biosystems) to amplify 60- to 150-bp amplicons. Amplicon was measured by SYBR Green fluorescence (SYBR Green Master mix, Applied Biosystems) in 10-µl reactions. Reactions were performed in triplicates. The amount of product was determined relative to a standard curve of input chromatin. Melting curves showed that PCRs yielded single product. Ct values for each sample were normalized against that of input DNA and percentage recovery is plotted. The primers used for QPCR will be available upon request.

Western Blotting, RT-PCR and miRNA Microarray

Whole-cell extracts were prepared, and approximately 10 μg of proteins were resolved on SDS-PAGE using antibodies against REST (#07-579, Upstate), c-myc (#sc-764, Santa Cruz Biotechnology), Oct4 (#ab19857, Abcam), Sox2 (#ab15830, Abcam), Actin (#sc-1616, Santa Cruz), and α-Tubulin (#MMS-407R, Covance) per the manufacturers' recommendations. Total RNA was extracted using TRIzol (Invitrogen), and approximately 50 ng of total RNA was used as a template. RT-PCR was performed using a OneStep RT-PCR kit (Qiagen).

The following gene-specific primers were designed: REST forward, 5′-AGCGAGTACCACTGGAGGAA-3′; REST reverse, 5′-CTGAATGAGTCCGCATGTGT-3′; Calbindin forward, 5′-GCTTCTATCTGGCGGAAGG-3′; Calbindin reverse, 5′-

TGTCATCTGGCTACCTTCCC-3′; Pitx2 forward, 5′-CGGCAGAGGACTCATTTCAC-3′; Pitx2 reverse, 5′-GTACGAATAGCCGGGGTACA-3′; Snai1 forward, 5′-CTTGTGTCTGCACGACCTGT-3′; Snai1 reverse, 5′-CTTCACATCCGAGTGGGTTT-3′, Sox18 forward, 5′-AACAAAATCCGGATCTGCAC-3′; Sox18 reverse, 5′-CGAGGCCGGTACTTGTAGTT-3′; Cebpa forward, 5′-CCGACTTCTACGAGGTGGAG-3′; Cebpa reverse, 5′-TGGCCTTCTCCTGCTGTC-3′. The following primers were based on published sequences: Gapdh, Oct4, Nanog, Gata6, Fgf5, brachyury, Flk1, and Pl1 [4]; Mash1, Mash2, β-globin, CD34, Cxcl12, Ehox, Eomes, Gata4, Isl1, Meox1, Mixl1, Ngn2, Shh, and Tbx3 [5].

For quantitative RT PCR, first strand synthesis was performed using Applied Biosystems' high capacity cDNA reverse transcription kit (cat. No. 4368814). Real time PCR was carried out using cDNAs with SYBR Green PCR master mix from Applied biosystems (cat. No. 4309155). Reactions were carried out in triplicates using ABI prism 7500 and the analysis was performed similar to that described for quantitative ChIP PCR above except that Δct was obtained after normalization with ct of Gapdh for mouse specific primers and that of Actin for human specific primers. Mouse specific primers will be provided upon request and human specific primers are as published in [6].

For the miRNA microarrays, total RNA was prepared using TRIzol, and RNA samples were analyzed by LC Sciences on their microarray with a probe set based on Sanger version 9.0. Multiple sample analysis involves normalization, data adjustment, t-Test/ANOVA analysis, and clustering. Normalization is carried out using a cyclic LOWESS(Locally-weighted Regression) method. The normalization is to remove system related variations, such as sample amount variations, different labeling dyes, and signal gain differences of scanners so that biological variations can be faithfully revealed. Data adjustment includes data filtering, Log2 transformation, and gene

centering and normalization. The data filtering removes genes (or miRNAs) with (normalized) intensity values below a threshold value of 32 across all samples. The Log2 transformation converts intensity values into Log2 scale. Gene centering and normalization transform the Log2 values using the mean and the standard deviation of individual genes across all samples using the following formula:

Value = [(Value) – Mean (Gene)]/[Standard deviation(Gene)]

In certain cases, expression ratios of dual-sample arrays are used in clustering analysis. Gene normalization would then be performed using the following formula:

Value = [Log2 (Ratio)] / [Standard deviation (Log2 (Ratio))]

t-Test is performed between “control” and “test” sample groups with each group contains at least two samples. T-values are calculated for each miRNA, and p-values are computed from the theoretical t-distribution. miRNAs with p-values below a critical p-value (typically 0.01) are selected for cluster analysis. The clustering is done using hierarchical method and is performed with average linkage and Euclidean distance metric.

ANOVA (Analysis of Variance) is an extension of the t-test to more than two experimental conditions. It picks out miRNAs that have significant differences in means across three or more groups of samples, with each group containing at least two samples. In this analysis, p-values are computed from the F-distribution. In certain cases, such as time point experiments, in which only one sample is collected and assayed for each condition or time point, repeating sets of reporters (or probes) on each array may be used as a “group” in an ANOVA analysis.

All data processes, except clustering plot, are carried out using in-house developed computer programs. The clustering plot is generated using TIGR MeV (Multiple Experimental Viewer) software from The Institute for Genomic Research.

miRNA validation was carried out using Ambion's *mir*Vana^{TM} qRT-PCR miRNA detection kit (Cat. # AM1558). mirVana qRT-PCR primer sets for the miRNA of interest were also obtained from Ambion (Cat. #30000–30999). Reactions were performed as per manufacturer's recommendations. The values obtained were normalized with primer set corresponding to 5S rRNA (cat. # AM 30302).

RNAi-Mediated Knockdown, exogenous expression of miR-21, anti-miR-21 and Gain-of-Function Experiments

RNAi-mediated knockdown of REST and Oct4 were carried out per the manufacturer's protocol (Dharmacon). siREST (60pmol), siOct4 (60 pmol), and nontargeting siRNA (60 pmol) were transfected using Amaxa nucleofection method. Colonies were assayed for alkaline phosphatase activity 3d after transfection.

Amaxa nucleofection was also used to deliver precursor miR-21 and anti-miR-21 molecules into mES cells. Transfection was carried out using 100 pmoles of precursor or 50 pmoles of miR-21 and anti-miR-21 each using $4x10^6$ cells. Non-targeting siRNA was used as control. Delivery and mature miR-21 product was inspected in cells by quantitative RTPCR 1d after transfection as described above.

For the gain-of-function experiments, mESCs were transfected with 2 μg of plasmid DNA using Lipofectamine 2000. The Flag-REST construct was a generous gift from G. Mandel. The transfected cells were selected with G418 (125 μg ml^{-1}) under self-renewing conditions in the presence of LIF for 3 d. After selection, we grew the cells under differentiation conditions in the absence of LIF for 4 days, and subjected them to self-renewal assays. An alkaline phosphatase assay was then performed using an alkaline phosphatase detection kit (Chemicon) after 4d. Percentages of self-renewing colonies from three independent experiments were plotted. Standard deviation was calculated and is depicted as error bars in the figures.

Immunofluorescence Microscopy of Blastocysts

Blastocysts from C57BL/6 NCr mice were fixed in 4% paraformaldehyde, permeabilized with 0.25% Triton X-100, and blocked with 1% goat serum in PBS [7]. The blastocysts were then double immunostained with mouse anti-REST antibody (raised against N-terminal portion of human REST, A372) in combination with antibodies against Sox2 (Abcam, cat # AB15830), Oct4 (Abcam, cat. # AB19857), Nanog (Abcam, Cat # AB21603) followed by Alexa Fluor 488-conjugated secondary antibody detecting mouse IgG and Fluor 555-conjugated secondary antibody detecting rabbit IgG (Molecular Probes, Carlsbad, CA). Images were captured with a Nikon microscope (Nikon Eclipse TE 2000U).

Discussion

Role of REST in the ICM of blastocyst

The role of REST in the ICM of the developing mouse blastocyst is still unclear. *REST*$^{+/-}$ haploinsufficient mice have an apparently normal phenotype[8], similar to that in mice haploinsufficient in most of the other known self-renewal factors or regulators, such as Oct4[9], Nanog[10], and Sox2[11], and the components of the LIF-STAT3 pathway (LIF[12], LIF-receptor β [LIFRβ][13] gp130[14], and STAT3[15]). All of these haploinsufficient mice were apparently normal. However, the complete loss of the individual regulators had variable effects. *REST*$^{-/-}$ embryos could survive past the blastocyst stage and showed progressive embryonic lethality between embryonic days 9.5 and 11, indicating an apparent lack of its role in regulating self-renewal and pluripotency of the ICM cell population under normal conditions[8]. In comparison, *Oct4,*$^{-/-}$ *Nanog,*$^{-/-}$ and *Sox2*$^{-/-}$ embryos developed to the blastocyst stage, but the ICM cells failed to produce normal

development[9-11, 16]. In contrast, embryos deficient in the components of the LIF-STAT3 pathway showed an apparent lack of an immediate effect on the ICM cells. *LIF*$^{-/-}$ mice were viable and showed only retarded postnatal growth[12], *LIFR*$\beta^{-/-}$ mice died only after birth[13], *gp130*$^{-/-}$ mice died between 12.5 days postcoitum and term[14], and *STAT3*$^{-/-}$ embryos developed until embryonic day 6 and then degenerated between embryonic days 6.5 and 7.5[15]. Thus, in terms of ICM development, *REST*$^{-/-}$ embryos are more similar to those deficient in the LIF-STAT3 pathway than to those deficient in Oct4, Nanog, and Sox2.

Although the exact nature of REST's role in the pluripotent ICM of a blastocyst is still unknown, there are several explanations. One is that the ICM cell population under normal conditions is transient, so that even the complete absence of REST does not produce its full detrimental effect. However, if the ICM cells were subjected to slowed development ("diapause"), a phenomenon that has evolved in some mammals, including mice, to get around adverse conditions during pregnancy, then the deficiency of REST would result in the developmental arrest of blastocysts. This last view is supported by the finding that *gp130*$^{-/-}$ blastocysts were arrested only when they were subjected to diapause[14].

References

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Supplementary Fig. S1

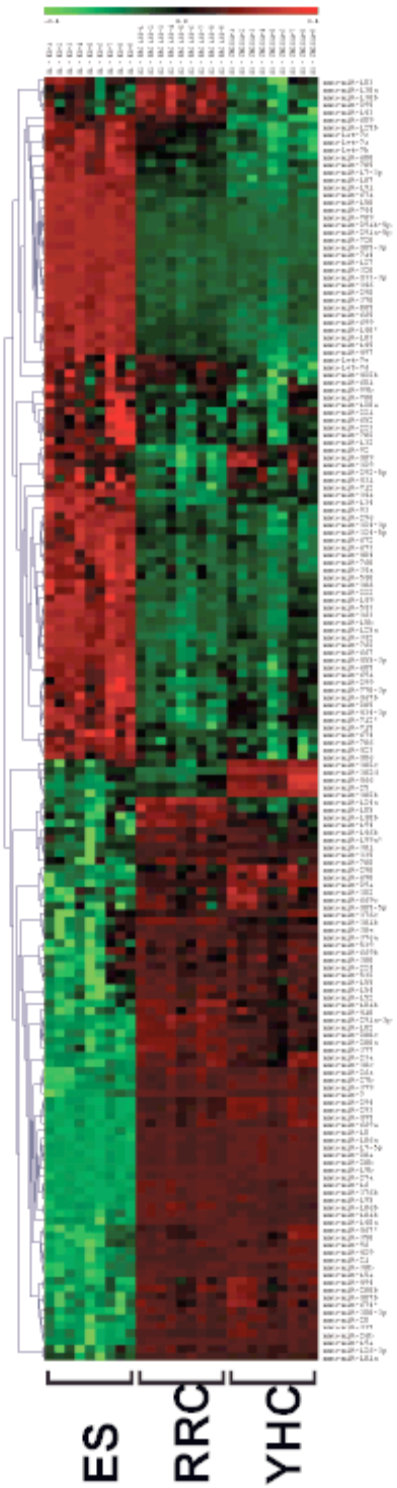

Table 1.

No.	Reporter Name	p value	S01 - ES Averaged Signal	S02 - RRC 160 Averaged Signal	S03 - YHC334 Averaged Signal
1	mmu-let-7a	1.09E-10	222	134	89
2	mmu-let-7b	5.90E-10	159	70	52
3	mmu-let-7c	2.73E-10	265	165	115
4	mmu-let-7d	6.88E-05	115	106	67
5	mmu-let-7d*	5.06E-01	1	34	38
6	mmu-let-7e	1.25E-05	194	146	99
7	mmu-let-7f	1.89E-01	46	44	37
8	mmu-let-7g	5.65E-03	1	53	44
9	mmu-let-7i	3.78E-01	1	41	38
10	mmu-miR-1		1	1	1
11	mmu-miR-100		1	26	1
12	mmu-miR-101a	4.63E-05	1	252	192
13	mmu-miR-101b	0.00E+00	1	561	467
14	mmu-miR-103	0.00E+00	2,857	1,260	996
15	mmu-miR-106a	0.00E+00	3,550	10,441	10,874
16	mmu-miR-106b	0.00E+00	1,217	3,097	2,764
17	mmu-miR-107	3.33E-16	2,745	1,148	860
18	mmu-miR-10a	1.59E-01	1	26	30
19	mmu-miR-10b	4.17E-01	1	1	1
20	mmu-miR-122a	2.68E-01	1	32	1
21	mmu-miR-124a	1.05E-12	253	491	281
22	mmu-miR-125a	4.66E-15	2,142	974	1,088
23	mmu-miR-125b	7.21E-11	520	369	236
24	mmu-miR-126-3p	1.62E-06	1	131	125
25	mmu-miR-126-5p	1.10E-01	1	1	1
26	mmu-miR-127	0.00E+00	5,573	2,541	2,419
27	mmu-miR-128a	8.72E-04	243	206	196
28	mmu-miR-128b	6.87E-02	216	199	189
29	mmu-miR-129-3p	2.42E-02	74	41	54
30	mmu-miR-129-5p	5.19E-01	1	1	1
31	mmu-miR-130a	6.84E-07	2,377	2,702	2,099
32	mmu-miR-130b	8.21E-10	1,362	1,672	1,216
33	mmu-miR-132	1.63E-04	101	56	55
34	mmu-miR-133a	7.13E-01	1	24	29
35	mmu-miR-133a*	7.75E-01	1	1	1
36	mmu-miR-133b	9.69E-01	1	23	1
37	mmu-miR-134	1.07E-08	1,189	733	865
38	mmu-miR-135a	6.68E-01	1	1	1
39	mmu-miR-135b	5.15E-01	1	1	1

40	mmu-miR-136	1.66E-01	1	1	1
41	mmu-miR-137	8.14E-01	1	1	1
42	mmu-miR-138	7.84E-01	1	1	1
43	mmu-miR-139		1	1	1
44	mmu-miR-140		1	1	1
45	mmu-miR-140*	0.00E+00	991	260	179
46	mmu-miR-141		1	78	60
47	mmu-miR-142-3p		1	25	29
48	mmu-miR-142-5p		1	1	31
49	mmu-miR-143	7.34E-05	87	107	70
50	mmu-miR-144		1	1	1
51	mmu-miR-145	0.00E+00	515	141	98
52	mmu-miR-146	2.98E-02	1	36	40
53	mmu-miR-146b	2.34E-04	1	64	58
54	mmu-miR-148a	1.11E-16	1	589	546
55	mmu-miR-148b	3.04E-06	1	181	122
56	mmu-miR-149	1.88E-11	291	143	147
57	mmu-miR-150	0.00E+00	3,387	1,324	1,202
58	mmu-miR-151		1	29	34
59	mmu-miR-152	1.23E-06	1	74	73
60	mmu-miR-153		1	1	1
61	mmu-miR-154	1.24E-10	177	333	313
62	mmu-miR-155	7.79E-06	1	87	71
63	mmu-miR-15a	1.11E-15	1	640	610
64	mmu-miR-15b	0.00E+00	11,298	6,257	6,409
65	mmu-miR-16	0.00E+00	2,439	8,717	7,742
66	mmu-miR-17-3p	5.59E-13	228	103	76
67	mmu-miR-17-5p	0.00E+00	4,846	16,377	17,673
68	mmu-miR-18	0.00E+00	279	1,505	1,619
69	mmu-miR-181a	3.92E-01	85	58	57
70	mmu-miR-181a*		1	1	1
71	mmu-miR-181b	4.14E-11	94	370	241
72	mmu-miR-181c	4.85E-01	1	51	42
73	mmu-miR-182	0.00E+00	3,776	7,403	6,455
74	mmu-miR-183	7.93E-04	4,679	4,636	4,165
75	mmu-miR-184	6.95E-01	1	1	1
76	mmu-miR-185	3.27E-05	238	316	262
77	mmu-miR-186	1.35E-02	1	41	33
78	mmu-miR-187	5.89E-01	1	45	42
79	mmu-miR-188	5.96E-01	1	26	1
80	mmu-miR-189	2.13E-01	1	1	1
81	mmu-miR-190	4.51E-01	1	18	1
82	mmu-miR-191	0.00E+00	5,946	2,641	2,077

83	mmu-miR-192	9.40E-01	1	1	1
84	mmu-miR-193	2.64E-02	1	1	1
85	mmu-miR-194	1.46E-09	1	91	58
86	mmu-miR-195	0.00E+00	269	1,365	1,331
87	mmu-miR-196a		1	1	31
88	mmu-miR-196b		1	1	31
89	mmu-miR-199a		1	1	29
90	mmu-miR-199a*	9.75E-04	1	72	62
91	mmu-miR-199b		1	1	1
92	mmu-miR-19a	3.33E-16	1	1,453	1,314
93	mmu-miR-19b	0.00E+00	696	10,463	10,411
94	mmu-miR-200a	2.22E-15	107	448	314
95	mmu-miR-200b	1.29E-11	823	1,287	1,252
96	mmu-miR-200c	8.36E-14	372	751	695
97	mmu-miR-201		1	28	1
98	mmu-miR-202	6.59E-01	1	29	26
99	mmu-miR-203		1	28	1
100	mmu-miR-204		1	1	29
101	mmu-miR-205	1.37E-07	142	68	82
102	mmu-miR-206	6.97E-01	1	25	1
103	mmu-miR-207	4.56E-03	48	25	28
104	mmu-miR-208	7.15E-01	1	1	1
105	mmu-miR-20a	0.00E+00	3,362	16,664	17,935
106	mmu-miR-20b	0.00E+00	2,850	13,239	14,518
107	mmu-miR-21	0.00E+00	1	4,561	7,097
108	mmu-miR-210	8.73E-01	84	83	86
109	mmu-miR-211	8.60E-01	1	1	26
110	mmu-miR-212	8.66E-01	1	1	36
111	mmu-miR-214	5.98E-03	109	25	1
112	mmu-miR-215	2.04E-01	1	1	1
113	mmu-miR-216a	5.80E-01	1	1	28
114	mmu-miR-216b	6.78E-01	1	1	25
115	mmu-miR-217	5.70E-01	1	1	1
116	mmu-miR-218	2.92E-02	1	39	38
117	mmu-miR-219	5.01E-01	1	1	1
118	mmu-miR-22	3.02E-03	275	244	216
119	mmu-miR-221	6.17E-04	370	281	312
120	mmu-miR-222	1.46E-10	434	250	257
121	mmu-miR-223	8.84E-05	72	37	27
122	mmu-miR-224	6.05E-04	1	136	136
123	mmu-miR-23a	1.99E-12	2,948	4,418	4,280
124	mmu-miR-23b	3.52E-02	4,186	4,612	4,331
125	mmu-miR-24	1.07E-02	1,611	1,464	1,635

126	mmu-miR-25	2.38E-12	12,055	14,126	18,825
127	mmu-miR-26a	4.11E-15	2,391	5,488	5,116
128	mmu-miR-26b	2.63E-12	94	612	777
129	mmu-miR-27a	0.00E+00	384	1,484	1,520
130	mmu-miR-27b	1.10E-14	1,078	2,009	2,055
131	mmu-miR-28	4.05E-13	79	215	222
132	mmu-miR-290	4.16E-10	28,814	32,641	36,170
133	mmu-miR-291a-3p	0.00E+00	7,491	16,422	13,050
134	mmu-miR-291a-5p	0.00E+00	14,771	4,565	4,215
135	mmu-miR-291b-3p	6.51E-01	1	1	33
136	mmu-miR-291b-5p	0.00E+00	16,083	5,109	4,644
137	mmu-miR-292-3p	1.91E-02	32,087	31,936	34,452
138	mmu-miR-292-5p	1.89E-04	30,601	27,358	28,703
139	mmu-miR-293	0.00E+00	21,562	38,942	43,016
140	mmu-miR-294	0.00E+00	26,417	50,248	58,993
141	mmu-miR-295	8.70E-06	22,402	24,412	21,067
142	mmu-miR-296	2.13E-10	257	106	94
143	mmu-miR-297	1.39E-03	1	68	62
144	mmu-miR-297b	1.61E-01	1	46	47
145	mmu-miR-298	0.00E+00	953	119	118
146	mmu-miR-299	7.86E-09	815	566	636
147	mmu-miR-29a	5.69E-11	341	626	780
148	mmu-miR-29b	9.54E-01	1	30	1
149	mmu-miR-29c	5.94E-01	1	1	1
150	mmu-miR-300	1.02E-06	178	265	275
151	mmu-miR-301	3.71E-04	1	511	393
152	mmu-miR-301b	2.98E-06	1	154	92
153	mmu-miR-302	5.98E-03	1	32	198
154	mmu-miR-302b	2.99E-10	1	53	638
155	mmu-miR-302b*	5.38E-01	1	1	1
156	mmu-miR-302c	4.01E-05	1	1	109
157	mmu-miR-302c*	3.29E-02	1	28	50
158	mmu-miR-302d	0.00E+00	400	304	1,453
159	mmu-miR-30a-3p	5.02E-02	1	73	66
160	mmu-miR-30a-5p	7.84E-03	427	491	505
161	mmu-miR-30b	6.66E-16	291	1,373	1,388
162	mmu-miR-30c	7.31E-13	1,246	2,156	2,033
163	mmu-miR-30d	8.54E-14	775	412	423
164	mmu-miR-30e	3.58E-05	1	217	186
165	mmu-miR-30e*	9.31E-01	1	1	1
166	mmu-miR-31	4.04E-03	235	274	219
167	mmu-miR-32	8.14E-01	1	1	1
168	mmu-miR-320	0.00E+00	1,323	309	304

169	mmu-miR-322		1	30	1
170	mmu-miR-323	5.70E-03	745	632	771
171	mmu-miR-324-3p	4.17E-14	224	52	38
172	mmu-miR-324-5p	7.78E-12	192	68	40
173	mmu-miR-325		1	1	1
174	mmu-miR-326		1	1	1
175	mmu-miR-328	1.09E-01	1	25	1
176	mmu-miR-329	2.99E-05	1,121	925	1,155
177	mmu-miR-33		1	1	1
178	mmu-miR-330	1.97E-01	1	1	1
179	mmu-miR-331	2.25E-03	1	1	1
180	mmu-miR-335	6.50E-05	1	274	388
181	mmu-miR-337	0.00E+00	199	658	652
182	mmu-miR-338		1	1	1
183	mmu-miR-339	1.57E-01	1	1	1
184	mmu-miR-340	5.47E-01	1	1	1
185	mmu-miR-341	0.00E+00	1,442	136	280
186	mmu-miR-342	1.11E-16	1,977	603	846
187	mmu-miR-344	9.35E-01	1	38	42
188	mmu-miR-345	8.85E-02	74	37	29
189	mmu-miR-346	0.00E+00	1,581	29	31
190	mmu-miR-34a	1.38E-10	927	655	616
191	mmu-miR-34b	8.65E-01	1	1	1
192	mmu-miR-34c	7.61E-01	1	1	28
193	mmu-miR-350	4.11E-11	105	276	306
194	mmu-miR-351	1.52E-03	99	44	55
195	mmu-miR-361	2.02E-02	1,038	1,180	1,103
196	mmu-miR-362	1.05E-01	1	34	47
197	mmu-miR-363	3.87E-13	2,762	1,629	1,763
198	mmu-miR-365	1.98E-01	44	25	29
199	mmu-miR-367	8.55E-01	1	1	1
200	mmu-miR-369-3p	2.36E-01	1	25	35
201	mmu-miR-369-5p	2.65E-03	1	50	60
202	mmu-miR-370	0.00E+00	512	105	98
203	mmu-miR-374-3p	7.60E-01	1	1	1
204	mmu-miR-374-5p	5.18E-03	1	118	242
205	mmu-miR-375	3.88E-01	1	33	39
206	mmu-miR-376a	5.14E-04	1	84	67
207	mmu-miR-376a*	8.16E-01	1	24	1
208	mmu-miR-376b	0.00E+00	1,046	4,988	4,568
209	mmu-miR-376b*	1.78E-02	1	37	45
210	mmu-miR-376c	2.74E-05	1	144	189
211	mmu-miR-377	8.85E-11	82	176	139

212	mmu-miR-378		1	1	1
213	mmu-miR-379	0.00E+00	865	2,512	2,705
214	mmu-miR-380-3p	7.16E-14	1	206	204
215	mmu-miR-380-5p	1.13E-01	1	57	53
216	mmu-miR-381	1.77E-02	149	152	170
217	mmu-miR-382	7.08E-04	1,099	1,250	1,281
218	mmu-miR-383	5.37E-01	1	1	1
219	mmu-miR-384		1	1	1
220	mmu-miR-409	5.38E-07	2,530	2,119	2,572
221	mmu-miR-410	1.32E-08	172	273	256
222	mmu-miR-411	4.51E-01	1	1	1
223	mmu-miR-412	5.66E-01	1	34	26
224	mmu-miR-422b	7.30E-05	227	201	155
225	mmu-miR-423	8.07E-07	91	26	1
226	mmu-miR-424		1	33	38
227	mmu-miR-425	6.58E-02	1	24	1
228	mmu-miR-429	0.00E+00	116	457	500
229	mmu-miR-431	3.92E-04	348	272	300
230	mmu-miR-433-3p	0.00E+00	2,279	472	476
231	mmu-miR-433-5p	1.14E-03	1	65	50
232	mmu-miR-434-3p	5.65E-09	3,070	1,896	2,293
233	mmu-miR-434-5p	1.05E-02	135	170	165
234	mmu-miR-448	6.92E-01	1	1	1
235	mmu-miR-449	2.80E-01	1	1	1
236	mmu-miR-449b	3.82E-01	1	1	1
237	mmu-miR-450	1.26E-02	1	44	44
238	mmu-miR-450b	8.49E-01	1	1	1
239	mmu-miR-450b*	7.94E-01	1	1	1
240	mmu-miR-451	1.79E-02	87	21	24
241	mmu-miR-452	4.13E-01	1	1	25
242	mmu-miR-455-3p	1.43E-11	150	37	42
243	mmu-miR-455-5p	7.76E-01	1	21	1
244	mmu-miR-463	5.85E-01	1	1	1
245	mmu-miR-464	5.82E-01	1	1	1
246	mmu-miR-465	8.25E-01	1	24	1
247	mmu-miR-466	3.21E-07	517	535	915
248	mmu-miR-467*	2.01E-13	106	539	725
249	mmu-miR-467a	1.71E-02	322	270	323
250	mmu-miR-467b	5.84E-09	129	33	40
251	mmu-miR-468	3.47E-01	1	21	1
252	mmu-miR-469	1.85E-01	1	1	1
253	mmu-miR-470	1.54E-01	41	36	31
254	mmu-miR-471		1	1	1

255	mmu-miR-483	1.17E-02	1	26	1
256	mmu-miR-484	1.11E-14	573	162	164
257	mmu-miR-485-3p	0.00E+00	5,185	460	409
258	mmu-miR-485-5p	8.37E-05	70	104	109
259	mmu-miR-486	2.03E-04	51	1	1
260	mmu-miR-487b	7.35E-13	324	577	638
261	mmu-miR-488		1	1	1
262	mmu-miR-488*		1	1	1
263	mmu-miR-489	7.14E-01	49	44	45
264	mmu-miR-490		1	1	1
265	mmu-miR-491	3.25E-01	1	1	1
266	mmu-miR-494	1.56E-11	1,706	2,593	2,838
267	mmu-miR-495	0.00E+00	1,452	3,257	3,526
268	mmu-miR-496	7.49E-01	1	49	42
269	mmu-miR-497	1.33E-15	186	52	39
270	mmu-miR-499		1	1	1
271	mmu-miR-500	1.71E-01	1	29	1
272	mmu-miR-501	7.28E-01	1	27	31
273	mmu-miR-501*	1.83E-01	1	48	50
274	mmu-miR-503	2.41E-01	1	1	28
275	mmu-miR-505	7.64E-01	1	1	1
276	mmu-miR-532	4.36E-06	1	154	159
277	mmu-miR-539	1.58E-07	118	206	205
278	mmu-miR-540	5.29E-09	165	93	86
279	mmu-miR-541	1.52E-01	4,717	4,581	4,488
280	mmu-miR-542-3p	5.36E-01	1	1	1
281	mmu-miR-542-5p	1.21E-01	1	1	1
282	mmu-miR-543	1.11E-16	4,234	2,098	2,344
283	mmu-miR-546	2.28E-01	1	1	1
284	mmu-miR-547	7.90E-01	1	1	1
285	mmu-miR-551b	7.50E-01	1	1	1
286	mmu-miR-592	9.21E-01	1	1	1
287	mmu-miR-615	8.07E-01	1	1	1
288	mmu-miR-652	9.42E-05	135	77	77
289	mmu-miR-665	0.00E+00	651	139	117
290	mmu-miR-666	3.19E-01	1	32	1
291	mmu-miR-667	1.11E-16	351	57	70
292	mmu-miR-668	1.12E-03	1	34	32
293	mmu-miR-669a	0.00E+00	732	1,715	1,813
294	mmu-miR-669b	1.87E-04	1	109	115
295	mmu-miR-669c	1.15E-07	461	711	814
296	mmu-miR-670		1	1	1
297	mmu-miR-671	0.00E+00	1,468	270	172

298	mmu-miR-672	1.04E-10	3,355	2,104	2,046
299	mmu-miR-673	1.90E-12	155	38	34
300	mmu-miR-674	2.17E-09	758	518	466
301	mmu-miR-674*	7.91E-10	166	271	278
302	mmu-miR-675-3p		1	1	1
303	mmu-miR-675-5p		1	1	1
304	mmu-miR-676	4.62E-03	1	89	69
305	mmu-miR-676*		1	1	1
306	mmu-miR-677	2.64E-01	1	1	1
307	mmu-miR-678	3.42E-01	1	1	1
308	mmu-miR-679	1.07E-01	1	37	41
309	mmu-miR-680	4.44E-16	861	506	340
310	mmu-miR-681	3.57E-04	1	27	1
311	mmu-miR-682		1	1	1
312	mmu-miR-683		1	1	1
313	mmu-miR-684	4.60E-01	1	1	1
314	mmu-miR-685	2.74E-09	125	31	35
315	mmu-miR-686	1.55E-01	1	20	1
316	mmu-miR-687	4.30E-01	1	1	1
317	mmu-miR-688		1	1	1
318	mmu-miR-689	6.42E-13	2,785	2,507	1,177
319	mmu-miR-690	8.25E-11	15,518	21,332	23,323
320	mmu-miR-691	2.04E-07	94	1	1
321	mmu-miR-692	8.99E-01	1	1	1
322	mmu-miR-693-3p	6.93E-01	1	1	1
323	mmu-miR-693-5p	5.10E-01	1	1	1
324	mmu-miR-694	8.02E-01	1	1	1
325	mmu-miR-695	4.27E-01	1	1	1
326	mmu-miR-696	1.99E-01	1	1	1
327	mmu-miR-697	9.75E-01	1	1	1
328	mmu-miR-698	6.01E-01	1	1	1
329	mmu-miR-699	0.00E+00	453	54	40
330	mmu-miR-7	9.48E-14	1	2,439	1,843
331	mmu-miR-700	6.97E-06	95	31	1
332	mmu-miR-701	9.08E-01	1	1	1
333	mmu-miR-702	3.57E-06	54	24	1
334	mmu-miR-703	4.88E-01	1	1	1
335	mmu-miR-704	9.17E-01	1	1	1
336	mmu-miR-705	3.98E-12	18,014	12,842	11,621
337	mmu-miR-706	1.09E-07	67	1	1
338	mmu-miR-707		1	1	1
339	mmu-miR-708	1.86E-04	1	56	40
340	mmu-miR-709	0.00E+00	66,303	19,741	17,945

341	mmu-miR-710		1	1	1
342	mmu-miR-711	2.45E-03	266	251	198
343	mmu-miR-712	1.29E-06	102	61	72
344	mmu-miR-712*	5.49E-11	145	54	79
345	mmu-miR-713	2.18E-03	1	1	1
346	mmu-miR-714	0.00E+00	5,224	719	741
347	mmu-miR-715	3.23E-07	71	1	1
348	mmu-miR-717		1	1	1
349	mmu-miR-718		1	1	1
350	mmu-miR-719	6.01E-01	1	1	1
351	mmu-miR-720	0.00E+00	10,572	687	600
352	mmu-miR-721	2.90E-01	1	1	1
353	mmu-miR-744	0.00E+00	13,741	5,525	5,141
354	mmu-miR-758	3.30E-01	75	72	83
355	mmu-miR-759	8.91E-01	1	1	1
356	mmu-miR-760	6.01E-13	161	34	36
357	mmu-miR-761		1	1	1
358	mmu-miR-762	3.33E-16	39,443	26,663	27,437
359	mmu-miR-763		1	1	1
360	mmu-miR-764-3p	3.04E-01	1	1	1
361	mmu-miR-764-5p	2.02E-02	1	1	1
362	mmu-miR-770-3p	3.12E-08	75	1	29
363	mmu-miR-7b	1.75E-02	1	1	1
364	mmu-miR-801	2.26E-01	1	58	82
365	mmu-miR-802		1	1	1
366	mmu-miR-804	6.96E-01	1	1	1
367	mmu-miR-805	0.00E+00	2,454	963	858
368	mmu-miR-9	3.63E-01	1	1	1
369	mmu-miR-9*	6.99E-01	1	1	1
370	mmu-miR-92	4.37E-14	25,069	17,680	23,035
371	mmu-miR-93	9.24E-12	4,829	3,554	3,437
372	mmu-miR-96	2.08E-11	1	453	406
373	mmu-miR-98	3.14E-01	1	1	1
374	mmu-miR-99a	8.51E-01	1	1	1
375	mmu-miR-99b	7.29E-04	1,167	978	928

Note

p values less than 0.01 are marked in Red
miRNAs having empty p value cells are of low signals

Table 2. Predicted miRNA target sites in known self-renewal genes (http://cbio.mskcc.org/mirnaviewer and http://pictar.bio.nyu.edu/)

Gene	Predicted miRNA target sites
Sox2	miR-145,miR-324-5p,miR-92, miR-129, miR-133a, miR-133b, miR-21, miR-182, miR-108, miR-320
Nanog	miR-27a, miR-21,miR-129, miR-182, miR-108, miR-9*, miR-320
Oct4	None
c-Myc	miR-34c, miR-34b, let-7c, let-7i, let-7b, let-7a, let-7f,miR-98, miR-302b*
Tbx3	miR-152, miR-106b, miR-106a, miR-17-5p, miR-20, miR-204, miR-30e-3p, miR-30a-3p, miR-153, miR-93, miR-372, miR-140, miR-206, miR-32, miR-299, miR-216

miRNA highlighted in red are common in both the databases.

Table 3. REST binding on predicted target sites using MatInspector module of Genomatix software. A representative of bound and unbound sites are shown in the table below.

miRNA	Sequence(s) (red: ci-value* > 60 capitals: core sequence)	Strand	Position relative to start site	REST binding
miR-21 site1/2	ctcAGCAcctgggagccagag atgAGCActaaggacagacga	(-) (-)	7214 bp upstream 7100 bp upstream	Bound
miR-21 site 4/5	gacAGCActggaggcaggtac atctaaaccCGGAgagctcc	(-) (-)	2453 bp upstream 2277 bp upstream	Not bound
miR-26a site 3	agcAGCAgcagatacagcagt	(-)	5559 bp upstream	Bound
miR-26a site 7	ataaagcccaCGGGcccagcc	(-)	717 bp downstream	Not bound
miR-27a site 7	ctcAGCAcctgtcacaactac	(-)	1840 bp upstream	Bound
miR-27a site 1	agaAGCAgcagggtcaggagt	(-)	9201 bp upstream	Not bound
miR-93/miR-106b site 7	agcaagtcccCGGAgagcaag	(-)	3708/3500 bp upstream	Bound
miR-93/miR-106b site 1	ggcAGCAgaagggacaggaga	(+)	9576/9368 bp upstream	Not bound
miR-152 site 1	tccAGCAccagcttcggtccc	(-)	9857 bp upstream	Bound
miR-152 site 9	ctcAGGAcctctggaagagca	(+)	8586 bp downstream	Not bound

* The **Ci-vector** (consensus index vector) for the matrix represents the degree of conservation of each position within the matrix. The maximum **Ci-value** of 100 is reached by a position with total conservation of one nucleotide, whereas the minimum value of 0 only occurs at a position with equal distribution of all four nucleotides and gaps.